PROCESSES FOR INHIBITING DEVELOPMENT OF ALLERGIC DISEASE

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FIELD OF THE INVENTION

The present invention is directed to processes for inhibiting development of allergic disease, particularly in mammals and birds. More specifically, the invention is directed to processes for inhibiting development of allergic disease by treatment of a neonatal or immature mammal or bird.

BACKGROUND OF THE INVENTION

A major health issue in the western world is the increase in allergic (atopic) diseases, e.g. asthma and allergies. It has been estimated that the incidence of asthma and allergies affects from about 15 to 30% of the population and continues to increase, with a major impact on health and productivity. Currently available remedies are typically primarily aimed at short-term treatment via antihistamines and topical nasal steroids. Preventive measures have generally not been very successful or well tolerated, e.g. allergy shots to common allergens. Accordingly, the need exists for additional preventive or inhibitive measures.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide novel means for inhibiting allergy development. It is a further object to provide improved means for

inhibiting allergy which overcome various disadvantages of prior art allergy treatments.

These and additional objects are provided by the present invention. In one embodiment, the invention is directed to a process for inhibiting development of allergic disease. The process comprises exposing a neonatal or immature mammal or bird to irradiation-detoxified lipopolysaccharide derived from microbial, protozoan and/or fungal endotoxin.

Additional embodiments of the invention are described in further detail below. The present inventors have discovered that safe application of lipopolysaccharide (LPS) into the natural environment for young children or animals, currently living in an "overly sterilized" environment, helps to restore the development of normal immune functions and inhibits the development of allergies later in life. Additional objects, embodiments and advantages of the invention will be more fully apparent and understood in view of the following detailed description.

DETAILED DESCRIPTION

The present invention is directed to processes for inhibiting development of allergic disease. The processes comprise exposing a neonatal or immature mammal or bird to irradiation-detoxified lipopolysaccharide derived from microbial, protozoan and/or fungal endotoxin. Within the context of the present specification, the term "inhibiting" encompasses both decreasing the development and, in certain embodiments, preventing the development, of allergy. Additionally, within the context of the present specification, the term "immature" refers to a mammal or bird which has not completed life cycle development to its adult stage. Further, within the

context of the present specification, the term "allergic disease" encompasses allergic atopic disease, e.g., both allergies and asthma.

It has been recently reported that growing up on a farm versus the modern urban "sterile" environment protects against allergic sensitization as well as the development of childhood allergic disease. See, for example, Ernst, P. et al, *Am J Resp Crit Care Med*, 161: 1563-1566 (2000), Gereda, J.E. et al, *JAMA*, 284: 1652-1653 (2000), and Gehring, U. et al, *Am J Respir Crit Care Med*, 166:939-944 (2002). It appears that regular contact with farm animals confers a protective effect. The protective effect of this environment has been linked to increased concentrations of endotoxin from gram-negative bacteria, which seem to stimulate the immune system. See, for example, Braun-Fahrlander, C. et al, *N Engl J Med*, 347:869-877 (2002) and Martinez, F.D., et al, *Lancet*, 354 suppl 2: SII 12-15 (1999). A microbial burden has been suggested to be crucial during the first years of life for developing a non-atopic, protective immune response, Matricardi, P.M., et al, *Allergy*, 58: 461-471 (2003). The cell components from bacteria, which are primarily responsible for activating the immune response are the lipopolysaccharides (LPS). However, LPS have undesirable side effects such as pyrogenicity, hypotension, and the like which typically prevent or at least limit their use as an immune stimulant, Thorn, J., *Inflamm. Res.*, 50: 254-261 (2001). Füst et al., *Infect Immun.*, 16: 26-31 (1977) have demonstrated that irradiation of LPS decreases their toxicity. Bertók et al. found that detoxified LPS retain some of their beneficial properties in animal experiments, *Immunopharmacology*, 8: 13-17 (1984), and, furthermore, irradiated LPS are not toxic as tested in human volunteers, *Orvosi Hetilap,* 140: 819-827 (1999).

The present inventors have now determined that irradiated LPS, versus native LPS, have significantly less stimulatory effect on Il-1 production of the cells in peripheral heparinized blood, as set forth in Table 1.

Table 1

In vitro comparison of the interleukin 1 (IL-1) released by native endotoxin (LPS) and irradiation-detoxified LPS (RDTX-LPS) from heparinized human peripheral blood cells.

CELLS	IL-1 (pg/ml) released (mean + SD)
Non-stimulated (control) cells	9.12 + 8.6
Cells stimulated with native LPS	172.26 + 26.7
Cells stimulated with RDTX-LPS	98.22 + 19.4

(Average values of 3 experiments. Measurement of IL-1 by ELISA in the supernatants). It should be noted that the values of SD for native LPS stimulated cells compared to SD values for RDTX-LPS stimulated cells are different for $p < 0.001$.

Accordingly, it is advantageous to inactivate the detrimental components of LPS derived from microbial (including bacterial), protozoan or fungal sources from a farm or other natural origin and then to apply the detoxified LPS fraction for stimulation of the Th-1 arm of the immune system. The safe application of LPS into the environment for young children or animals, for example, mammals or birds, currently living in an "overly sterilized" environment, will help to restore the development of normal immune function and prevent or decrease the development of allergies later in life.

In a more specific embodiment, the present processes provide a safe, irradiation detoxified LPS to young mammals in order to stimulate an immune response by stimulating the Th-1 arm of the immune system, thereby inhibiting, by preventing or minimizing, the development of allergic (atopic) disease such as

common allergies and asthma. More specifically, the present process through irradiation inactivates the undesirable effects of the endotoxins from microbes, including bacteria, protozoan or fungi and while retaining their immune stimulatory antiallergenic properties. In one embodiment, the endotoxins are extracted from bacteria, protozoan or fungi. In a more specific embodiment, the endotoxins are extracted from bacterial, protozoan or fungal sources in a farming or other natural environment. In a further embodiment, the endotoxins are extracted from, for example, *E. coli*.

Any suitable extraction method known in the art may be employed, an example of which is a solvent extract system, preferably with polarity adjustment to maximize yield. Such solvent extraction systems may employ a phenol-water system, other alcohol-water systems, an acetonitrile-water system, or the like. The extracted endotoxins may then be irradiated with sufficient radiation to detoxify the toxins therein, for example radiation in an amount of from about 25 to about 150 kGy. The irradiation changes the structure of the endotoxin, removing toxicity, while maintaining its positive immune effect. Typically, the endotoxins may be irradiated in an aqueous solution. The neonatal or immature mammal or bird is exposed to the resulting detoxified LPS, whether through environmental exposure or by direct contact with the animal. As detailed below, exposure can be effected by various means, inducing the long-lasting stimulation of the immune system without any dangerous side-effects.

In one embodiment, the subject is an infant human. In a further embodiment, the subject is a human of 1 month to 2 years of age. In an alternate embodiment, the subject is a primate of 2 weeks to 12 months of age. In yet further embodiments, the subject is a dog or cat of 1 week to 12 months of age. In additional embodiments, the

subject is a farm animal, for example a cow, pig, goat, horse, chicken or turkey of 2 days to 12 months of age.

The exposure may be achieved by various means. In one embodiment, a topical composition comprising the irradiation-detoxified lipopolysaccharide is applied to the subject. Any topical composition vehicle may be employed. For example, in one embodiment, the topical composition further comprises a powder vehicle or carrier, examples of which include, but are not limited to talcum powder, corn starch, beet starch, rice flour, oatmeal, or a mixture thereof. In an alternate embodiment, the topical composition is in the form of a topical cream, lotion or gel. Examples of suitable cream, lotion or gel base components include, but are not limited to, water, short and long chain alcohols, acids, esters and oils, alkylene glycols, glycerols, glycerides, petroleum jelly and the like. The compositions may also include additional components typically employed in topical compositions, for example, vitamins, fragrances, herbal extracts, humectants and the like.

The exposure may be achieved by contacting the subject with a cloth or film impregnated with, for example, a suspension or dispersion of the irradiation-detoxified lipopolysaccharide. Any type of woven or nonwoven natural or synthetic cloth may be employed. Suitably, the irradiation-detoxified lipopolysaccharide may be formulated in a water or water/alcohol base for impregnation. More specifically, the subject can be contacted with a wipe impregnated with a composition comprising the irradiation-detoxified lipopolysaccharide. In a further embodiment, an infant can be provided with a diaper impregnated with a composition comprising the irradiation-detoxified lipopolysaccharide.

Alternatively, the exposure may be achieved by administering an aerosol spray composition comprising the irradiation-detoxified lipopolysaccharide to surrounding

environment or directly to the subject. Such a spray may contain an aqueous, organic or mixed vehicle and may be sprayed from a pressurized or manual pump spray container, in accordance with spray techniques known in the art. The aerosol spray may then be provided in a living area to achieve the desired exposure.

The exposure level should be that sufficient to obtain the desired inhibition effects. In a specific embodiment, the irradiation-detoxified lipopolysaccharide is delivered in a concentration from 0.01 ug/g to 100 ug/g of delivery vehicle, for example, topical composition, impregnated cloth, or aerosol spray. In a further embodiment, exposure to the irradiation-detoxified lipopolysaccharide is achieved shortly after birth and during the maturing life cycle of the mammal or bird. In a more specific embodiment, exposure to the irradiation-detoxified lipopolysaccharide is achieved on a daily or weekly basis during growth of the mammal or bird.

The following examples illustrate non-limiting specific embodiments of the compositions suitable for use in the processes of the invention.

Example 1

This example demonstrates one embodiment for preparation of irradiated LPS. Other methods will be apparent to one of ordinary skill in the art and are equally suitable for use in connection with the present inventive processes.

From a fermented culture of *Escherichia coli* 0101 H, LPS is extracted by a modified "hot-phenol-water" method according to Westphal et al, *Z. Naturforsch.*, 76:148-155 (1952). The resulting powdered LPS is suspended in distilled water (10 mg/ml) and irradiated in the cabin of NORTOM equipment (with cooling) by ^{60}Co gamma irradiation (150 kGy). The irradiated material is lyophilized immediately. The detoxification of LPS is measured using a biological test in animals for residual toxicity and for endotoxin tolerance inducing capacity. The irradiated LPS

preparation can preserve its biological activity for about 10 years in the lyophilized form.

Example 2

This example demonstrates a pressurized aerosol formulation:

Sterile Water	100 ml
Irradiated LPS	10 ug/ml

Butanol or Nitrogen (propellant) sufficient for aerosol delivery

Example 3

This example demonstrates a manual pump aerosol formulation:

Sterile Water	100 ml
Irradiated LPS	15 ug/ml

Example 4

This example demonstrates another manual pump aerosol formulation:

5% isopropyl alcohol	100 ml
Irradiated LPS	5 ug/ml

Example 5

This example demonstrates a dry powder formulation:

Talcum powder	100 grams
Dried irradiated LPS	20 ug/gram talcum powder

Example 6

This example demonstrates another dry powder formulation:

Corn starch	100 grams
Dried irradiated LPS	25 ug/gram corn starch

Example 7

This example demonstrates another dry powder formulation:

Beet starch and rice flour	100 grams
Dried irradiated LPS	5 ug/gram beet starch

Example 8

This example demonstrates another dry powder formulation, particularly advantageous for application to a subject via bath water:

Oatmeal	100 grams
Dried irradiated LPS	5 ug/gram oatmeal

Example 9

A cloth such as a diaper is provided with irradiated LPS applied uniformly as powder in an amount of about 0.01 ug. In another embodiment, a diaper is provided with irradiated LPS applied uniformly as a liquid in an aqueous carrier in an amount of about 0.01 ug.

In a further embodiment, a single wipe cloth is impregnated with irradiated LPS in an amount of about 0.01 ug/wipe.

Example 10

This example demonstrates a topical cream or lotion formulation:

A base mixture of purified water, canola oil, stearic acid, glycerol stearate, propylene glycol, glycerin, cetyl alcohol, lecithin, aloe vera gel, squalane (vegetable), avocado oil, vitamins A, D and E, D-panthenol, allantoin, chamomile extract, herbal fragrance, propylparaben, methylparaben, and triethanolamine, is combined with irradiated LPS in an amount of about 10 ug irradiated LPS per 50 ml of the base mixture.

Example 11

This example demonstrates another topical cream or lotion formulation:

Petroleum jelly	100 grams
Irradiated LPS	0.1 ug/gram petroleum jelly

Example 12

This example demonstrates another topical cream or lotion formulation:

Base mixture of sweet almond oil, shea butter and vitamin E

Irradiated LPS	2.5 ug per gram base mixture

The specific illustrations and embodiments described herein are exemplary only in nature and are not intended to be limiting of the invention defined by the claims. Further embodiments and examples will be apparent to one of ordinary skill in the art in view of this specification and are within the scope of the claimed invention.